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T.A.Slotkin, S. Pachman, J. Bartolome, R.J. Kavlock

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Department of Pharmacology
Duke University Medical Center
Durham, NC 27710

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16. ABSTRACT

Administration of methylmercury (1 or 2.5 mg/kg daily) to neonatal rats caused alterations in both cardiac and renal growth patterns. Heart weights were elevated in the preweaning period in association with hyperplasia (supranormal DNA content); after weaning, the cardiac overgrowth regressed and there was an eventual hypoplasia as evidenced by low DNA content in young adulthood. Renal overgrowth was more pronounced and persistent, but reflected a pure hypertrophy, with no changes in DNA. Despite the absence of apparent morphologic damage to the kidney, renal function was affected by neonatal methylmercury exposure, as assessed through basal clearance techniques. In the immediate period after beginning treatment, there was an impairment of renal function (elevated serum urea, creatinine and osmolality; increased fractional excretions of water, sodium and osmotic particles), with a return to normal by 10 days postnatally. Thereafter, there was a secondary phase of tubular impairment which peaked at the time of maximum hypertrophy. Thus, biochemical and functional indices of organ development can be adversely affected at doses of methylmercury which are usually associated primarily with nervous system-specific damage; effects of methylmercury on neuronal and/or hormonal factors may contribute to the perturbations.

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BIOCHEMICAL AND FUNCTIONAL ALTERATIONS IN RENAL AND CARDIAC DEVELOPMENT RESULTING FROM NEONATAL METHYLMERCURY TREATMENT

THEODORE A. SLOTKIN, STEVEN PACHMAN, JORGE BARTOLOME and ROBERT J. KAVLOCK[a]

Department of Pharmacology, Duke University Medical Center, Durham, NC 27710 and [a]Health Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, NC 27711 (U.S.A.)

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SUMMARY

Administration of methylmercury (1 or 2.5 mg/kg daily) to neonatal rats caused alterations in both cardiac and renal growth patterns. Heart weights were elevated in the preweaning period in association with hyperplasia (supranormal DNA content); after weaning, the cardiac overgrowth regressed and there was an eventual hypoplasia as evidenced by low DNA content in young adulthood. Renal overgrowth was more pronounced and persistent, but reflected a pure hypertrophy, with no changes in DNA. Renal function was affected by neonatal methylmercury exposure, as assessed through basal clearance techniques. In the immediate period after beginning treatment, there was an impairment of renal function (elevated serum urea, creatinine and osmolality; increased fractional excretions of water, sodium and osmotic particles), with a return to normal by 10 days postnatally. Thereafter, there was a secondary phase of tubular impairment which peaked at the time of maximum hypertrophy. Thus, biochemical and functional indices of organ development can be adversely affected at doses of methylmercury which are usually associated primarily with nervous system-specific damage; effects of methylmercury on neuronal and/or hormonal factors may contribute to the perturbations.

Key words: Methylmercury; Development; Kidney; Heart; DNA; Renal function

Address all correspondence to: Dr. T. Slotkin, Box 3813, Duke Univ. Med. Ctr., Durham, N.C. 27710, U.S.A.

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INTRODUCTION

Methylmercury is now found ubiquitously and represents a major environmental hazard. Nowhere is this more apparent than in the developing organism, where methylmercury exerts a variety of deleterious effects both in the fetus and the neonate. Because of its devastating effects on the central and peripheral nervous system, methylmercury is most often regarded as a developmental neurotoxin and indeed, even at very low doses behavioral alterations are prominent [reviews, 1,2]. This substance also achieves high concentrations in a variety of non-neuronal tissues, such as kidney, liver and heart which may then also be potential targets [3—5]. At high doses, methylmercury causes frank morphological damage to these organs with a consequent deterioration of function [1—8]. Recent studies indicate, however, that subtle alterations in renal and cardiac development may occur even at low-level exposures to methylmercury: for example, the ontogenetic pattern of ornithine decarboxylase, an enzyme which serves as a major control point for cellular replication, differentiation and growth [reviews, 11—13], was profoundly affected in hearts and kidneys of developing rats exposed to doses of methylmercury which would ordinarily be associated almost exclusively with neurobehavioral actions [9,10]. These findings raised the possibility that methylmercury could produce damage which might not be apparent by classical morphological criteria but which nevertheless could compromise functional development of affected organs. In the current study, we have examined the effects of postnatal methylmercury exposure on cardiac and renal development, using dosage regimens which span the threshold for neurochemical and behavioral damage [1,2,9,10,14,15]. To assess the consequences of alterations in biochemical variables (such as ornithine decarboxylase), DNA content was measured in each tissue; additionally, since the kidney may be a specific target for organomercurials, a variety of functional tests were conducted to evaluate whether performance capabilities of renal tissue could be compromised.

METHODS

Timed pregnant Sprague—Dawley rats (Zivic-Miller Laboratories, Zelienople, PA) were housed individually in breeding cages and allowed food and water ad libitum. Pups from all litters were randomized at birth and redistributed to the nursing mothers, with litter sizes kept at 10 or 11 pups to maintain a standard nutritive status. Randomization within each treatment group was carried out at intervals of several days and in addition, pups of both sexes were chosen for each experiment from several different cages for each group; experimental groups were always sex-matched to the controls. Neonates were given methylmercury hydroxide (methylmercury (II) hydroxide as a 1 M solution in water, obtained from Alfa Products, Danvers, MA) at a daily dose of 1 or 2.5 mg/kg s.c., beginning the day after birth and continuing until weaning (21 days); this regimen has been previously

shown to be effective in producing neurobehavioral damage without compromising survival or causing generalized signs of toxicity [1,2,9,10,14,15]. Controls received daily injections of water equal in volume to the methylmercury injections (1 ml/kg). At intervals of several days, animals were weighed and selected for either renal function tests or for cardiac and renal DNA determinations. Extraction and analysis of DNA was carried out as described in detail in a previous study [16].

Renal function tests

On each day of observation, a basal clearance test was applied to 7—10 rats in each treatment group. In this procedure, rats were isolated from fluid intake for a 3-h period and the urine produced during that span was collected quantitatively. Rats under 2 weeks of age are poikilothermic; consequently at those ages the animals were placed in beakers containing bedding and the beakers incubated in a water bath which maintained the bedding surface temperature at 32°C. Older animals were placed in metabolism cages to capture spontaneous voidings; until weaning, plastic beakers were fitted with funnels draining into small vials, whereas at 30 and 41 days of age standard Nalgene metabolism cages were used. To initiate the test, the bladders were voided using techniques described previously [17] and a urine sample saved for analysis of osmotic pressure. Immediately after the 3-h test period the bladders were again voided and urine collected quantitatively. Prior to day 10, trunk blood was collected after decapitation; thereafter, blood was drawn by external cardiac puncture under light CO_2 anesthesia. After separation of serum by centrifugation, aliquots of urine and serum were analyzed for chloride using a Radiometer CMT 10 chloride titrator, osmotic pressure using a Wescor 5100B Vapor Pressure Osmometer, and sodium, potassium, urea, creatinine and glucose using a Beckman ASTRA 4 Analyzer; urinary excretion of osmotic particles was determined from urine osmolality and volume. Clearance and fractional excretions were then calculated using standard formulae.

Statistics

Data are presented as means and standard errors as a percentage of control values, with significance calculated from the untransformed absolute values by two-way ANOVA (factor 1 = age, factor 2 = treatment). Differences were considered significant at a criterion of $P < 0.05$.

RESULTS

Methylmercury administration to developing rats had only minor effects on body weight at 1 mg/kg (Fig. 1); however, a significant deficit was apparent even preweaning in the group given 2.5 mg/kg. The general pattern of cardiac growth indicated an early phase in which relative heart weights tended to be elevated (higher than normal heart/body wt ratio); this was followed by a post-weaning deficit in which the shortfall in heart weight was

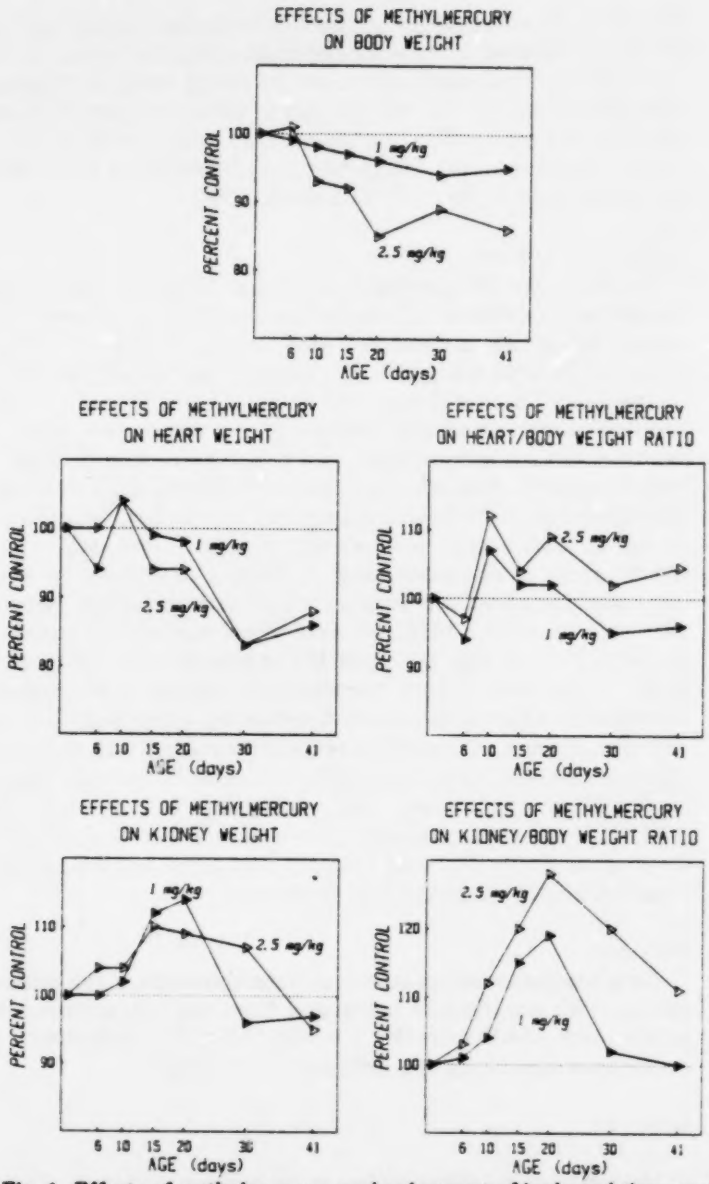

Fig. 1. Effects of methylmercury on development of body and tissue weights. Data represent means of values from 6 to 16 rats in each group at each age, presented as percentage of control values, which were: body weight (g), 16.5 at 6 days, 26 at 10 days, 36 at 15 days, 52 at 20 days, 109 at 30 days and 193 at 41 days; heart weight (mg), 81, 112, 166, 267, 404, 722; kidney weight (mg/pair), 206, 300, 416, 598, 1210, 1763. Standard errors were <6%. Two-way ANOVA indicates significant effects of methylmercury on all variables measured.

typically no greater than that in overall growth. A totally different pattern was apparent for growth of the kidney: there was no shortfall of renal growth on an absolute basis in either of the methylmercury groups and in fact there was a substantial overgrowth apparent on both an absolute or relative basis.

In order to understand the reasons for methylmercury-induced alterations in tissue weights, hearts and kidneys were examined for DNA content (Fig. 2). In the heart, the initial phase was associated with transient elevation of DNA content centered about 10 days of age; during the subsequent post-weaning deficit in tissue weight, there was an equivalent reduction in DNA content. In contrast, the DNA content of the kidney was relatively unaffected despite the significant increase in weight.

Renal function studies

Rat pups treated with methylmercury showed an acute impairment of tubular function, as evidenced by both serum chemistry (Fig. 3) and clearance and fractional excretion measurements (Fig. 4). Within 24 h of the first methylmercury injection, elevations were seen in serum urea, creatinine and osmolality, and in fractional excretions of water, sodium and total osmotic particles as well as in free water clearance. At this time, glomerular filtration appeared to be unimpaired, with little or no change in creatinine clearance; thus the effects on serum urea and creatinine probably reflected dehydration subsequent to the reduced tubular reabsorptive capacity. Evidence of adaptation or repair of tubular damage was noted over the ensuing few days and by 10 days of age most of the variables returned to normal. However, by the end of the second postnatal week, many of the indicators of renal function

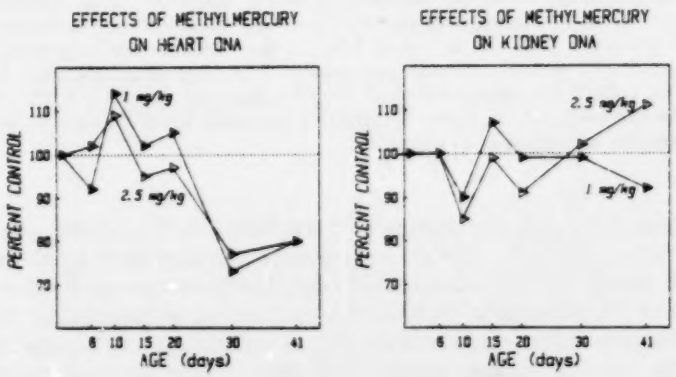

Fig. 2. Effects of methylmercury on development of heart and kidney DNA content. Data represent means of values from 6 rats in each group at each age, presented as percentage of control values, which were: heart (mg/organ), 0.35 at 6 days, 0.38 at 10 days, 0.57 at 15 days, 0.59 at 20 days, 0.89 at 30 days and 1.09 at 41 days; kidney (mg/pair), 1.24, 1.65, 2.71, 3.29, 5.20, 7.88. Standard errors were <7%. Two-way ANOVA indicates significant effect of methylmercury on heart DNA content but not kidney.

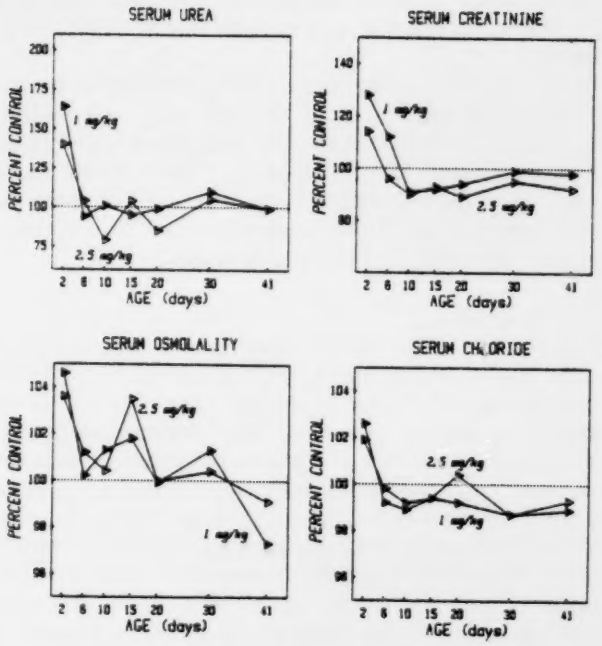

Fig. 3. Effects of methylmercury on serum chemistry. Data represent means of values from 7 to 10 rats in each group at each age, presented as percentage of control values, which were: 9.0, 8.5, 6.8, 6.5, 7.2, 4.5 and 4.9 mM for urea (S.E. <9%); 59, 56, 46, 50, 41, 51 and 51 μM for creatinine (S.E. <4%); 284, 286, 291, 296, 312, 304 and 310 mOsM for osmolality (S.E. <0.8%); and 88, 88, 88, 91, 97, 89 and 88 mM for chloride (S.E. <1%). Two-way ANOVA indicates significant age x treatment interactions for urea, creatinine and osmolality.

again indicated the presence of dysfunction. Fractional excretions of water, glucose, sodium, chloride and osmotic particles were all elevated, with a peak at about 20 days of age. Although this corresponds to the period in which renal hypertrophy reached its maximum, it should be noted that these changes reflect impairment, not enhancement of tubular function. During this second phase, serum urea and creatinine concentrations were not elevated in treated pups (in fact, they were generally reduced). Eventually, most of the markers returned to control levels, with the notable exception of creatinine clearance; because of difficulties in quantitative recovery of urine from the Nalgene metabolism cages, clearance values tended to be highly variable in animals postweaning and hence greater reliance should be placed on serum chemistry at 30 and 41 days.

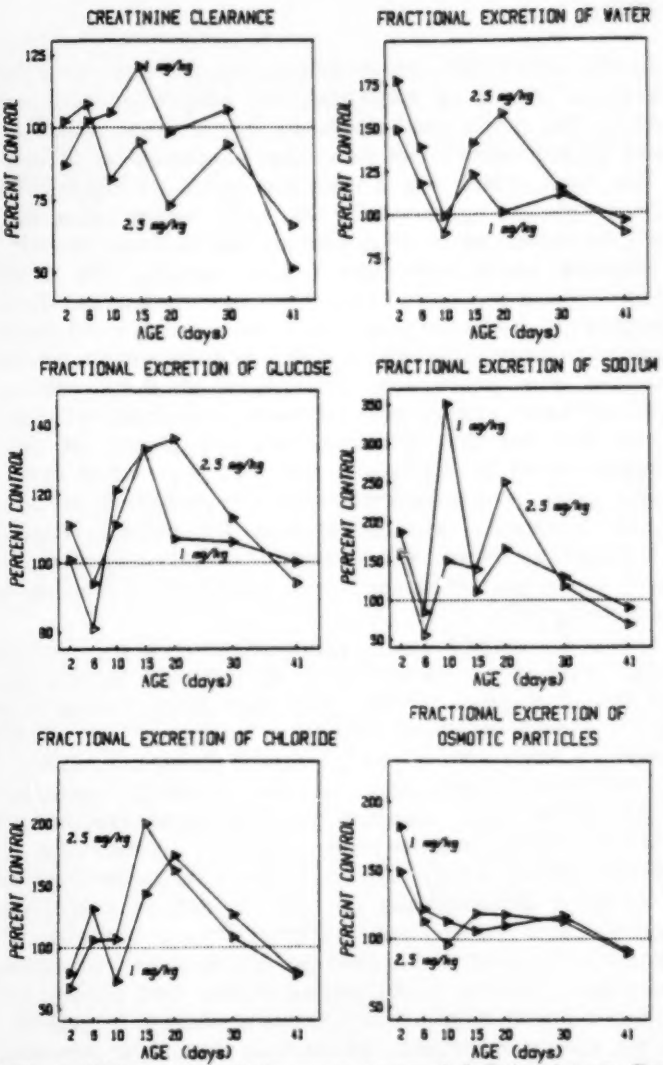

Fig. 4. Effects of methylmercury on renal functional tests. Data represent means of values from 7 to 10 rats in each group at each age, presented as percentage of control values, which were: 0.9, 1.0, 1.5, 1.7, 2.9, 3.5 and 4.4 μl/g/min for creatinine clearance (S.E. <12%); 4.0, 2.2, 2.0, 3.9, 1.4, 1.3 and 1.0 percent for fractional excretion of water (S.E. <17%); 2.0, 0.5, 0.2, 0.2, 0.1, 0.2 and 0.2% for fractional excretion of glucose (S.E. <10%); 0.2, 0.3, 0.1, 0.3, 0.2, 1.3 and 1.0% for fractional excretion of sodium (S.E. <33%); 2.5, 0.5, 0.3, 0.2, 0.5, 2.9 and 3.1% for fractional excretion of chloride (S.E. <33%); and 6.0, 4.7, 3.4, 4.7, 3.9, 5.5 and 5.3% for fractional excretion of osmotic particles (S.E. <9%). Two-way ANOVA indicates significant age × treatment interactions for creatinine clearance as well as for fractional excretions of water, sodium, chloride and osmotic particles.)

DISCUSSION

Results obtained in this study lend support to the view that developmental alterations caused by methylmercury involve multiple organ systems and further, that subtle biochemical damage serves to detect and predict subsequent growth-related and functional consequences of low-level exposures. In the heart, there was a transient period of higher-than-normal relative growth, typified by elevated heart/body weight ratios and, at its peak (10 days), by elevations in DNA content; this indicates an early phase of cardiac hyperplasia which terminates before weaning. The initial promotion of cardiac cellular replication should not be viewed as beneficial, however; after regression of the hyperplasia, there was a pronounced deficit in DNA which occurred in association with a deficit in heart weight. It is important to note that this later phase does not involve abnormal *relative* heart weights (although heart weights were decreased, heart/body wt ratio was not); nevertheless, the fact that the tissue was hypoplastic and not simply growth-impaired serves to distinguish this particular effect from that on general weight gains. It is noteworthy that the perturbations in cardiac DNA are entirely consistent with those seen for ornithine decarboxylase, since methylmercury causes early elevations of enzyme activity which peak in the first 2 weeks postnatally and then decline to normal or subnormal values postweaning [9].

Neonatal methylmercury exposure also caused increments in kidney weight, both on an absolute and relative basis. In this case, however, the alterations appear to reflect a pure hypertrophy, as no increments in DNA content were seen. Studies from our laboratory have established that renal overgrowth does involve the increase in RNA and protein which would be expected from hypertrophy and does not involve hydronephrosis [18,19]; in this earlier work, standard histocytological examination of the hypertrophied kidneys indicated no structural abnormalities [19]. Again, the increased kidney weight is predictable based on biochemical measurements of ornithine decarboxylase [9], which indicate a prolonged elevation of enzyme activity consequent to methylmercury exposure. What is especially important, however, is the clear-cut evidence for impairment of renal function which occurred in the treated groups. Two distinct phases of methylmercury-induced renal alterations were evident. In general, these reflected adverse effects on tubular, rather than glomerular function; initially, there was a diminished reabsorptive capacity of the tubules of sufficient magnitude to induce a state of dehydration and elevated serum osmolality. Although renal function and serum chemistry became normal within a few days of commencing methylmercury treatment, a secondary effect was seen (centered about day 20), and the possibility of more persistent or even later changes in tubular and glomerular function cannot be ruled out. Indeed, it should be emphasized that these alterations in renal function were detectable under basal physiological conditions; since the kidney is endowed with considerable functional reserve capacity, tests conducted at

the extremes of physiological challenge may reveal even greater impairment.

The delayed effect of methylmercury on renal function corresponds to a period of increased sensitivity to damage by inorganic mercury [20], and such functional alterations could involve mercurial intoxication per se. However, some of the methylmercury-induced alterations in both cardiac and renal development may reflect endocrine or nervous system-specific actions as well. Previous studies have shown that neonatal methylmercury exposure has profound effects on the ontogeny of sympathetic innervation of autonomic neuroeffector organs as well as increasing impulse flow in sympathetic pathways [10,18]. Because catecholamines from sympathetic neurons are responsible for terminating the period of active DNA synthesis in tissues such as developing heart [21,22], the promotion of sympathetic activity by methylmercury could, in fact, contribute to the subsequent hypoplasia noted in that tissue. Similar factors may be involved in the effects of methylmercury on renal growth and function. Renal norepinephrine rises most notably at the end of the second week of postnatal life [23] and the secondary phase of altered renal function occurs right at that time. Furthermore, renal reactivity to β-adrenergic input and to a variety of trophic hormones (including vasopressin) matures much earlier in methylmercury-treated neonates [18,19], a factor which would promote the actions of neural and endocrine activity on development of this tissue. As one demonstration of how such secondary factors can influence renal development, chemical sympathectomy with 6-hydroxydopamine has been shown to prevent the initial onset of renal hypertrophy [10].

In conclusion, neonatal exposure to relatively low doses of methylmercury produces both biochemical and functional alterations of cardiac and renal development. In the heart, there is an initial phase of overgrowth consequent to hyperplasia but after regression there is a shortfall in DNA characteristic of eventual hypoplasia. Renal overgrowth is also apparent, but involves hypertrophy rather than changes in cell number. Most importantly, methylmercury adversely affected renal performance, indicating that biochemical and functional measures can uncover subtle damage. In addition to direct effects of the mercurial on cardiac and renal development, some of these alterations may involve effects on neuronal and endocrine factors which help to regulate end-organ maturation and functional capacity.

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